Evaluation the Heart Failure Test in Heart Failure patients with Bacterial Infection

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ABSTRACT

Objective: To evaluate the parameters that effected by bacterial infection in heart failure patients.

Methods: The study was conducted at October-2020 to May-2021 in the Hospital of Imam Hussain Medical City in Karbala. About 10 mL of venous blood samples were taken from heart failure patients after admission to the CCU and control. There are important procedures of blood culturing we have to follow it after that many analyses had been conducted such as CRP, BNP and Troponin

Results: were CRP (46.013), BNP (218.84), Troponin (Heart Failure Without Bacterial Infection (924.526), and Heart Failure with Bacterial Infection 383.97) and Staphylococcus hominis was the most bacteria present.

Conclusion: Troponin was a useful diagnosis of heart failure and very important indicator for bacterial infection in Sepsis with heart failure. Moreover, Staphylococcus hominis was the most common genius in heart failure patients.

INTRODUCTION

Heart failure is a complex clinical syndrome that develops due to any functional or structural heart problem that impairs ventricular filling or ejection of blood into the systemic circulation to meet systemic demands. Endocarditis, myocarditis, pericarditis, heart valve disease, blood vessel disease, and metabolic problems can all induce heart failure. The majority of people with heart failure experience symptoms as a result of poor left ventricular myocardial function. Common symptoms include dyspnea, poor exercise tolerance, and fluid retention, characterized by pulmonary and peripheral edema. More than 6.2 million people in the United States and more than 23 million worldwide suffer from the disease. While this number of HF is expected to increase to 1.5 million annually by 2040. The most widely used terminology for defining HF is the left ventricular ejection fraction (LVEF). HF with preserved ejection fraction (HFrEF) is described as HF with normal LVEF (50%) and HF with reduced LVEF (40%) as HF with reduced ejection fraction (HFrEF). HF patients with an LVEF of 40% to 49% are classified as...
having mid-range ejection fraction (HFmrEF). Several lines of evidence have demonstrated that bacterial infections may play a major role in the etiology of CVD, whereas the other risk factor include diabetes, hypertension, hyperlipidemia, and smoking. Bacterial infections can induce CVD by directly or indirectly interacting with inflammatory and immunological mechanisms. Infection has been found to directly alter endothelium function via circulating endotoxins, stimulate smooth muscle cell proliferation and local inflammation, and trigger the innate immune response. Indirectly harmful effects of bacterial infections include the induction of pro-inflammatory, hypercoagulable, atherogenic responses, oxidation of low-density lipoprotein, antigen mimicry between bacterial, host cells, nutrient/vitamin malabsorption, and metabolic disruptions like excess ammonia production. Infections are a rising clinical concern that can cause heart failure (HF) decomposition as well as a life-threatening acute systemic illness (Sepsis) and septic shock in some situations. In sepsis and refractory septic shock, the circulatory system plays a crucial role in the development of multiorgan failure. Sepsis with cardiac dysfunction has a substantially higher fatality risk than sepsis without cardiac dysfunction. Infections are a common cause of HF decomposition, and if sepsis and/or septic shock are present, they should be identified early and treated with specialist methods.

**METHODS AND MATERIALS**

**Patients**

The case-control study of patients with heart failure was conducted during the period from October 2020 to May 2021. Seventy-one patients presenting with heart failure in the coronary care unit (CCU) in Heart Center of Al-Hussein Teaching Hospital, Al-Hussein Medical City, Kerbala, Iraq Health Directorate. All patients were adults and aged up to forty-fives (>45) and form both sexes were admitted as heart failure.

**Collection Samples**

About 10 mL of venous blood sample where taken from heart failure patients after admission to the CCU. There was an important procedure of blood culturing done by Nutusi et al. should be followed.

**Diagnosis of Bacteria**

**Blood Culture Samples**

Blood was taken from a peripheral vein and put into blood culture bottles. The BacT/ALERT® 3D system (bioMérieux, Marcy l'Étoile, France) was utilized to first examine the blood cultures. The bacteria were collected and inoculated on blood agar plates (BAP; Asan Pharmaceutical Co., Ltd., Seoul, Korea) and MacConkey agar plates (Becton Dickinson, Sparks, MD, USA), then incubated at 35°C in a 5% CO2 atmosphere.

**Conventional Workflow of Positive Blood Cultures**

Gram staining was performed after the BacT/ALERT® 3D Device gave a positive signal, followed by culturing on a suitable solid agar medium. The commercial automated VITEK® 2 system-generated colonies on the agar plates were used for identification and antibiotic susceptibility testing (AST) (bioMeriéux) after an overnight incubation period. The ID and AST values acquired utilizing this traditional approach were used for comparison as the institution's protocol.

- With a simple uniform inoculum, there is minimal handling after primary organism isolation.
- At the Smart Carrier StationTM, put the inoculum into the VITEK® 2 Cassette.
- The barcode connects the VITEK® 2 Card with the sample.
- The equipment will handle all further incubation and reading operations once the Cassette has been loaded.

VITEK® 2 Compact is an automated biochemical-based tool that includes 48 biochemical features and is widely used in clinical laboratories for microbial detection. Microorganisms can be identified for up to 4 hours using VITEK® 2 Compact. Each well assesses a strain's metabolic function, including its ability to acidify, alkalize, and enzymatically hydrolyze substrates and develop bacteria in the presence of inhibitors. In microwells, fluorescence-based sensors are employed to track bacterial growth and metabolic changes. The outcomes of biotyping and biochemical-based techniques may be influenced by the parameters of bacterial incubation, such as media composition or pH. A sterile microloop was used to collect a few colonies from a pure culture that had grown on blood or MacConkey agar for 18 to 24 hours. Bacterial culture was calibrated to the McFarland Turbidity Standard of 0.5–0.63 in 3 mL of 0.45 percent sodium chloride solution using a VITEK® 2 DensiChek (bioMérieux, Warszawa, Poland). The GN card was placed on the Cassette and placed in the instrument if the gram stain was negative. If the gram stain was positive, the GP card was placed on the Cassette and placed in the instrument. The time between suspension preparation and card filling was less than 30 minutes to avoid turbidity changes. At 35.5 1°C, the cards were incubated. Colorimetric measurements were taken automatically every 15 minutes when each card was taken out of the incubator. The results were read after 10 to 18 hours incubation.

**Identification of Heart Failure**

**Identification of Heart Failure by ECG and ECHO**

The specialist doctor made the identification of heart failure patients by ECG and ECHO in the CCU.

**Diagnosis of Heart Function Tests**

**Estimation BNP**

- The protocol was followed in the preparation of all reagents, standard solutions, and samples. Before use, all of the reagents were brought to room temperature. The assay is carried out at room temperature.
- The strips were inserted into the frames and used after determining the number of strips required for the assay. The unused strips should be stored between 2 and 8°C.
• In the standard well 50 micrometers of the standard were added.
• In sample wells 40 μL of sample A were added, followed by 10 μL of anti-BNP antibody in sample wells, and finally 50 micro liters of streptavidin-HPR in the sample and standard wells. I did a good job mixing. The plate was then sealed and incubated for 60 minutes at 37°C.
• The plate was washed five times with wash buffer after the sealer was removed. Soak wells with at least 0.35 mL for each shower and wash buffer for 30 seconds to 1 minute. To ensure automatic washing, aspirate both wells and wash five times with wash buffer, overfilling wells with wash buffer each time. Blot the plate with paper towels or another absorbent material. –
• Before incubating the plate filled with a fresh sealant at 37°C in the dark for 10 minutes, 50 (μL) of substrate solution A was applied to each well, followed by 50 (μL) of substrate solution B.
• The plate was incubated in the dark for 10 minutes at 37°C, with 50 μL of substrate solution A and 50 μL of substrate solution B in each well.
• Each well's optical density (OD value) was assessed using a microplate reader set to 450 nm within 10 minutes of adding the stop solution.

Estimation of Troponin
Influence the method of Alan et al.\(^1\)7

- Using a transfer pipette 75 μL of human serum were transferred to an empty sample mixing tube, and 75 μL of detection buffer were added.
- The sample's cover was closed over the mixing tube, and the sample was properly mixed by shaking it about 10 times.
- 759 μL were pipetted from the sample mixture and put into the sample well of the cartridge.
- At room temperature for 12 minutes, the sample-loaded cartridge was left out.
- For ichroma testing, the single-loaded cartridge was inserted into the instrument's cartridge holder. The cartridge was checked for proper orientation before being fully inserted into the cartridge holder.
- The "Select" button on the equipment was pressed to start the scanning procedure for ichroma samples.
- The ichroma testing instrument will begin scanning the sample-loaded cartridge automatically.
- The results of the ichroma measurements were read on the instrument's display screen.

Table 1: CRP mg/L of the study sample.

<table>
<thead>
<tr>
<th>Patients of Heart Failure</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>Mean ± SD mg/L</td>
</tr>
<tr>
<td>Without bacterial infection</td>
<td>45.4662 ± 57.545</td>
</tr>
<tr>
<td>With bacterial infection</td>
<td>43.478 ± 53.333</td>
</tr>
<tr>
<td>Total</td>
<td>46.013 ± 56.361</td>
</tr>
<tr>
<td>P value</td>
<td>0.9020</td>
</tr>
</tbody>
</table>

* means significance differences (P < 0.05) ** means high significances differences (P <0.001)

RESULTS AND DISCUSSIONS

CRP
The results of the statistical analysis of Table 1 showed that there was a highly significant increase (p < 0.001) in the mean of CRP in patients of heart failure compared to the control, as the mean of CRP for heart patients and the control (46.013 and 4.454) mg/L respectively. The results of the same statistical table also showed an insignificant decrease (p > 0.05) in the mean of CRP in patients of heart failure with bacterial infection compared to the heart failure without bacterial infection, as the rate of RDW-SV (43.478 and 45.4662) mg/L respectively.

This is similar to the previous studies that reported in patients with IE, elevated CRP levels at hospital admission and vegetation length at diagnosis were independent predictors of in-hospital death in patients with IE, even when other
Agreements with another study that reported traditional infection and inflammatory markers including procalcitonin, C-reactive protein, and leukocyte count, some heart failure markers such as the N-terminal pro-B-type natriuretic peptide (NTpro-BNP) have been proposed to help diagnose septic cardiomyopathy.\textsuperscript{22} According to a previous study involving 900 patients, NT-pro-BNP and high-sensitive cardiac troponin T (hsTNT) are strongly related to septic shock development (with or without septic cardiomyopathy). In another research, NT-pro-BNP surpassed hscTNT in predicting septic shock patients' 90-day mortality. NT-pro-BNP and hs-cTNT, on the other hand, failed to detect septic cardiomyopathy with appropriate specificity in that study.\textsuperscript{23} Agreements with the study that reported atrioturetic peptides are peptide hormones produced predominantly by the heart muscles in response to increased volume status and wall stress, both important aspects of cardiovascular physiology.\textsuperscript{24} BNP (brain natriuretic peptide) is a routinely measured natriuretic peptide that is released as the heart expands and the myocardial wall pressure rises. BNP's physiological functions include relaxation of vasomotor tone, suppression of sympathetic activity, reduction of cardiac preload, rise in renal blood flow, and increase in natriuresis and diuresis.\textsuperscript{25} In clinical decision-making, plasma BNP has become a useful tool for diagnosing, managing, and risk stratifying heart failure.\textsuperscript{26,27}

**Troponin**

The results of the statistical analysis of Table 4 showed that there was a highly significant increase (p < 0.001) in the concentration of Troponin in patients of heart failure without bacterial infection compared to the control, as the concentration of Troponin for heart patients without bacterial infection and the control 924.526 and 0.100 ng/mL respectively. And high significant increase (p < 0.001) in the results of the same statistical table in the concentration of Troponin in patients of heart failure with bacterial infection compared to the heart failure without bacterial infection, as the mean of BNP (197.48 and 226.11) ng/dL respectively.

This is an agreement with the study that there tests for detecting cardiac Troponin (cTn) in myocardial tissue that have high clinical sensitivity and specificity. Furthermore, due to diverse causes, many assays can detect cTn in the early stages...
of necrosis or even in the absence of necrosis (increased myocyte turnover or increased cell wall permeability, among others). Because of these characteristics, cTn has become the gold standard biomarker for myocardial damage and the optimal biomarker for detecting acute myocardial infarction.\textsuperscript{28} Furthermore, in the HF population, cTn levels are common. In up to 93 percent of patients with acute HF and up to 74% of patients with stable chronic HF, cTn concentrations are greater than the 99th percentile of the reference value.\textsuperscript{29} The researchers looked into the link between intubated patients with Sepsis who had a positive troponin level and the length of time they were on mechanical ventilation in the ICU. Similar to a recent study that found that 36% of individuals had high troponin levels, whereas Bessiere \textit{et al} estimated that roughly 61 percent of patients hospitalised with Sepsis had positive Troponin.\textsuperscript{30} It was identical to Sheyin \textit{et al}’s study (60.5 percent). Previous research on sepsis and septic shock had mixed results when it came to the link between troponin increase and clinical outcomes. Several studies including those by Vallabha-josyula \textit{et al} \textsuperscript{31} and Sheyin \textit{et al}\textsuperscript{32} have established that elevated troponin levels are linked to poor outcomes and higher mortality in patients admitted with Sepsis.\textsuperscript{33}

**Microbiological Tests**

**Bacteria Isolation**

From observation the results of table 4 that the most common genus in heart failure patients is \textit{Staphylococcus} and insignificance differences from other genres, while the most species heart failure patients is \textit{Staphylococcus hominis ssp hominis} in significance differences from other species.

Another study on patients with infective endocarditis with microbiological findings are: \textit{Streptococcus} sp., \textit{n (%) 12 (34.3%)} \textit{Streptococcus pyogenes}, \textit{n (%) 1 (2.9%)} \textit{Streptococcus agalactiae}, \textit{n (%) 1 (2.9%)} Non-haemolytic \textit{streptococci}, \textit{n (%) 10 (28.6%)} \textit{Staphylococcus sp.}, \textit{n (%) 9 (25.7%)} \textit{Staphylococcus aureus}, \textit{n (%) 2 (5.9%)} Negative coagulase \textit{staphylococci}, \textit{n (%) 7 (20.0%)} \textit{Enterococcus sp.}, \textit{n (%) 5 (14.3%)} \textit{Enterococcus faecalis}, \textit{n (%) 3 (8.6%)} \textit{Enterococcus faecium}, \textit{n (%) 1 (2.9%)} (34). Non-haemolytic \textit{streptococci} belonged to the following species: \textit{Streptococcus oralis, Streptococcus mutans, Streptococcus galloyticus, Streptococcus agalactiae, Streptococcus homans, Streptococcus bovis, Streptococcus parasanguinis}. Negative coagulase \textit{staphylococci} belonged to the following species: \textit{Staphylococcus epidermidis, Staphylococcus hominis and Staphylococcus warneri}.\textsuperscript{34}

### Table 4: Troponin (ng/mL) of the study sample.

<table>
<thead>
<tr>
<th>Infection</th>
<th>Mean ± SD ng/ml</th>
<th>Mean ± SD ng/ml</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without bacterial infection</td>
<td>924.526 ± 175.346</td>
<td>0.100 ± 0.00</td>
<td>0.0001 **</td>
</tr>
<tr>
<td>With bacterial infection</td>
<td>383.97 ± 57.446</td>
<td>0.100 ± 0.00</td>
<td>0.0001 **</td>
</tr>
<tr>
<td>Total</td>
<td>802.713 ± 157.934</td>
<td>0.100 ± 0.00</td>
<td>0.0001 **</td>
</tr>
</tbody>
</table>

* means significance differences (P < 0.05) ** means high significances differences (P < 0.001)

### Reference